In the second place, anthropological investigations go to show that of the fundamental primitive arts, pottery-making, for various obvious reasons, is of relatively late date in culture history, throughout the world. The archeology of the eastern United States seems particularly clear on this point. Thus, it has been demonstrated over and over again that the lower strata of artificial deposits from the Ozark uplift to the Atlantic coast and from lower New York state to Florida are devoid of ceramics. Narrowing the field to the east coast of Florida, we have on record several independent determinations (one by the writer only last spring and not yet published) to the effect that the shellmound people did not at first possess any pottery at all, that after a time they began making a plain dull-reddish earthenware, and that finally, some time before the arrival of European explorers, they took to ornamenting this ware by impressing upon it some simple geometric patterns.

Now pottery fragments, apparently of the undecorated variety, occur also in the Vero deposit, and the archeologist, rather than accepting an extraordinary hiatus in his own data, will be disposed to consider the section in which it was found to be synchronous with the middle period of the local shellmound occupation. To accept the Vero date at its present face value would compel him not only to relegate the development of pottery to an unheard of date but also it would oblige him to assume that this early culture of Pleistocene times was snuffed out and that after some millenniums marked by the arrival of the modern fauna a new and lower type of culture became established which only after a very considerable period reached the level of the original culture. Such a happening is conceivable, but it is not plausible.

So far as the writer can see, the archeologists can do very little more than they have done already toward the solution of the Vero problem. Extended investigation by an archeologist would in all probability yield nothing, because on the real points at issue he would always have to defer to the geologist and the paleontologist. If we could persuade the paleontologist to satisfy himself about the fauna of the shellheaps something might result. Errors of identification may have been made in the past. If he can close the gap between the shellmound fauna and that of the Vero section nobody will be happier than the passing generation of archeologists. But even then the complete solution will not have been reached because we shall still be facing a situation which appears to require one of two things: either the anthropologist must surrender not only his present lightly held opinion regarding the antiquity of man in America, but also his rather more firmly fixed notion regarding the order and progress of cultural traits in general, or else the paleontologist must concede us a very much narrower margin of time as having elapsed since the close of the Pleistocene than he has hitherto.

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THE AMERICAN MUSEUM OF NATURAL HISTORY

SPECIAL ARTICLES

A NEW METHOD FOR INVESTIGATION OF THE PERIPHERAL NERVOUS SYSTEM, MUSCLES AND GLANDS

IN preparing and preserving animals for investigation of the gross anatomy of the peripheral nervous system, muscles and glands, simple methods commonly in use have not proven very satisfactory.

For the study of anatomical structures alcohol does not differentiate sufficiently either to separate the parts from each other or from surrounding tissues. Aside from its cost, moreover, alcohol is open to the objection that it makes the parts brittle. Formalin has been used with better results and is now the standard means employed in preparing, and particularly in preserving, portions of the central nervous system. While both these reagents are preservatives of the peripheral nerves, muscles, and glands, neither is a satisfactory preparative for their dissection.

A successful fluid for this purpose should not only preserve, but it should also differentiate the anatomical systems from each other and bring to view the constituents of the

parts studied, with their relations to the other parts, without disturbing the natural mobility of the tissues as a whole. Such a fluid should reduce the work of dissection so that a minimum of disturbance is necessary in order to reach the parts under investigation.

A most serious objection to formalin is the adhesive effect it has upon tissues, so that parts are not readily separable. Muscles are stuck together in a more or less brittle or fused mass and the nice mobility of tissues observable in a fresh specimen is wholly destroyed. Resultant color changes, too, are such as to make dissection more difficult, for muscles are altered from their characteristic reddish to whitish tints, and it is consequently impossible or exceedingly difficult to trace the smaller nerves to regions of their ultimate distribution.

Formalin adds to, rather than subtracts from, the amount of dissection required, on account of the care necessary in avoiding severance of parts to be left intact.

After giving formalin a thorough trial as an aid for studying the peripheral nervous system it was discarded altogether and the use of fresh specimens employed as a substitute method. But while fresh material is much easier to work upon, the finer nerves are even less distinct than they are after the use of formalin. Moreover, fresh tissues soon begin to deteriorate and the animals become unfit for further use. Pieces of ice kept constantly near, or upon, the material were then tried, with some improvement. Although the animals can be kept for a period of three weeks by resorting to the ice chest, the dissections are not satisfactory. The reason for this is, not only because too much mechanical effort is necessary to segregate the parts, but also because the smaller nerves are not brought clearly to view.

A temporary preservative, and, what is of much more importance, an almost ideal preparative, for the investigation of the peripheral nervous system, muscles and glands, is found in hydrochloric acid.

The fresh animals may be first put in a 5 per cent. solution of *hydrochloric acid ice-water* and left for twenty-four hours. They may be skinned or not, as the problem in hand requires, but the body and chest cavities should in any case be opened to allow the fluid to penetrate through the tissues.

In preparing specimens for work on the cutaneous nerves it is necessary, of course, to leave undisturbed whatever portions of skin are to be studied; otherwise, it is best to remove the entire skin.

After treatment with hydrochloric acid the animals are washed in the coldest water obtainable from the faucet, and put in receptacles deep enough so that the material can be kept covered with ice-water or at least cold water. These receptacles are then stored in the refrigerator when the specimens are not in use.

In using animals so prepared it is found practical to wash them first in running water, leaving the pan partly filled, and then to add pieces of ice sufficient to surround the specimen while observation and dissection are going on. In tracing the smaller nerve divisions, details are brought out better if, occasionally, dilute acid is added, by means of a pipette, directly to the parts under consideration, since by this treatment the transparency of the muscle fibers is increased.

Guinea pigs treated by the above method were found to be in excellent condition for following medullated nerve fibers far into the tissues which they supply.

The 5 per cent. acid solution increases the whiteness of the nerves bringing them into sharp contrast with the natural reddish, or reddish-brown, back-ground of muscles, but if much stronger acids are used, even 10 per cent., it tends to whiten the muscles and dissolve the fibers without improving the color of the nerves.

Animals are also put in good condition for dissection if treated with a 6 per cent. acid solution. If a specimen is to be used during a long period it is better to give an initial twenty-four hour treatment in an acid solution not stronger than 3 per cent. and subsequent immersions in the same strength of acid for shorter periods. In any case the water left

upon the specimen while it is being kept in the refrigerator should be changed at least once a day.

However, the percentage of acid below 6 per cent. may be varied considerably. Indeed, 3 parts of acid to 1,300 parts of water has been found to bring to distinct view cutaneous and other nerves lying near the surface, if the animal is left in the solution three or four days. Interior parts, as the muscles of the eye, then appear reddish, but may be made almost transparent by addition of dilute acid directly to the dissected part as the head lies covered with cold water in the dissecting pan.

Doubtless several factors are involved, in deciding strength of acid to be used. Chief of these is the *size* of the animal, the *nature* of the tissue and the *location* of parts to be studied.

The use of this acid method for investigating the anatomy of various animals, together with the best means of preserving them over long periods, is under investigation and will be treated in a future paper.

In the 5 per cent solution muscles are not only separated from each other but the fiber bundles, of which they are composed, are brought out distinctly, by the breaking down of connective tissue between them. The entire muscle thus made more or less transparent, allows its smaller nerves to appear.

An excellent illustration of the advantage of this transparency was found in the case of the *orbicularis oculi*. After treatment in the acid solution, following the removal of the skin peripheral to the eyelids, little further dissection was needed for study. The orbital and palpebral portions of the muscle, their constituent fibers, with the ramifications and anastomoses of motor and sensory nerves within them, were distinctly observable throughout the breadth and depth of the muscle.

The effect on the muscles of the body wall is such as so to separate the constituent parts and so increase their transparency that the smaller divisions of the nerves within the muscle can be observed at various depths.

Action of the acid seems to continue after removal of the specimen from the solution. In the preliminary treatment, the blood vessels are easily followed and veins can be distinguished from arteries, while anastomoses of blood vessels upon the walls of the alimentary tract, for instance, are brought out clearly. But, as the action of the fluid (5 per cent. HCl) continues for some days, red corpuscles are gradually dissolved and the smaller vessels become less clearly discernible. After a week or so, the inner lining of the stomach wall is loosened from the outer layers of muscle and the latter is broken up into its longitudinal, circular and oblique fibers. Later stages of treatment show clearly the interlacing of the muscle fibers of the heart.

The skeletal parts are found, on removal from 5 per cent. acid, to be decalcified sufficiently to yield readily to cutting with scissors or breaking with forceps, so that the dissection of nerves where they pass through bony channels is rendered easy. On the whole the tissues are broken down so very gradually that a single specimen can be used for weeks.

The most fortunate feature of the method lies in the fact that connective tissue is the first to be seriously attacked by the acid. Much of it remains even to the later stages of dissolution, but appears less dense while its strands become so weak that it is readily separable from parts which it holds together. Nerves and muscles, on the other hand, are about the last of the soft parts to be broken down.

Small unmedullated sympathetic fibers, however, are not favorably affected for dissection by this method and consequently are not as easily traceable as are medullated fibers. That sympathetic fibers are not dissolved by the solution is certain, since the larger ones, and even a few of the smaller ones related to the blood vessels in the orbit, can be traced with accuracy for some distance. This method, therefore, cannot be recommended for study of the sympathetic system, other than of its grosser parts. In such investigations it is decidedly useful, in locating all the larger

ganglia in the body cavity and elsewhere, together with many of their gross connections.

This method has also been proved to be of advantage in the study of glands. Here, again, the breaking down of connective tissue seems to be the chief factor. Glands are thus separated from other organs, the outlines of their lobes come into view, ducts are released from their envelopes and the nerve supply, wherever medullated, can be easily traced. In the study of glands the color effects from this treatment, as in the case of nerves, are helpful to investigation.

It is apparent that a readily applied anatomical method which brings parts to distinct view with little or no dissection is of wide usefulness in embryology. A statement by Professor Mead of the applicability of this method to pig embryos will be found below.

One of the greatest advantages of the method, whether applied to nerves, muscles or glands, is finally to be mentioned, namely, it permits the use of the camera lucida for drawing. It has been found entirely practicable to mount a camera lucida (Abbé type) over the right eye-piece of a binocular microscope and to reduce the field of the left eye-piece by a superposed cylinder 1.8 cm. long, the upper aperture of which is 3 mm. in diameter. This arrangement prevents the observer from shifting the eye to a different view from the one desired and from thus throwing out of position the lines already drawn, as the work proceeds.

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Application of the Method to the Dissection of Pig Embryos

The method here described by Mr. Longwell has proved to be very valuable in preparing pig embryos for general dissection. Embryos from 12 mm. to nearly full term were treated with about 1 per cent. HCl and either kept for 2 or 3 days in the refrigerator or, when weather was cold, out of doors and sometimes frozen. In some cases they remained in the refrigerator for a month. Some of the specimens were dissected immediately after rinsing the acid off with water and others were kept in about 1 per cent, solution of carbolic acid.

The treated specimens retain nearly the texture and pliability that they had when taken from the uterus. The muscles are rendered slightly more opaque rather than transparent as in the guinea pigs prepared by Mr. Longwell. The nerves, therefore, do not present the striking contrast to muscles in color which his specimens show. In case of the embryos, however, the slight opacity of the muscles is rather an advantage.

The advantages of the method as applied to the embryos are the complete lack of rigidity or brittleness, the extraordinary ease with which the adjacent parts can be separated when the connective tissue is partly dissolved. The epithelium separates from the true skin and the latter from the superficial fascia with the greatest ease. The skin muscles, for example, the platysma and facial and auricular muscles, show with diagrammatic clearness. The deeper muscles retain sufficient strength for purposes of dissection. The nerves retain their strength entirely and are white. It is easy to follow them to their minutest branches. The cerebrospinal and sympathetic ganglia are also tough.

The facility that the method lends to the dissection of embryonic glands and ducts is equally delectable. The ducts of the submaxillary and parotid in a pig of 100 mm. can still be followed to their ultimate branches. The liver becomes soft but when pinched between the fingers and washed the branches of the vessels, the gall bladder, cystic and hepatic ducts, etc., are left and the relation of the omenta and the foramen of Winslow are most satisfactorily exposed.

Tendons, fascia, the peritoneum, blood vessels and meninges retain sufficient toughness for satisfactory dissection. The brain and cord are of better texture and color than in either fresh specimens or those prepared in formalin or fixing agents followed by alcohol.

In general Mr. Longwell's method is invaluable in the dissection of pig embryos.

A. D. Mead